# THE LONG-TERM EFFECT OF ELEVATED $CO_2$ ON GRASSLAND BIOMASS PRODUCTION

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November 1996

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**ABSTRACT**

This study investigates the influence of elevated $CO_2$ on grassland biomass production at a naturally elevated $CO_2$ spring situated on the Bongwan gas fault in Natal. The effect of elevated $CO_2$ on monocotyledenous ($C_4$) and dicotyledenous ($C_3$) above ground plant biomass production and their dominance patterns along a $CO_2$ gradient were studied. Three 7×7m plots were located 18m, 39m and 73m away from the elevated $CO_2$ spring. The 18m site was the experimental site, while the other two sites were the controls. The primary focus of the study was to determine the biomass production of monocotyledenous and dicotyledenous plants at the above-mentioned distance from the spring. However, to ascertain possible factors that could influence the increase in biomass production with distance from the $CO_2$ source, plant nutrient analyses (N and P), soil moisture contents (which could have an effect on plant water-use efficiency) and carbon isotope discrimination values were determined at the three sites. The results show that elevated $CO_2$ had a significant effect on the monocotyledenous dry matter production, but had no significant effect on any of the other plants or soil. It was also shown that elevated $CO_2$ increased the soil water retention capacity as one moves toward the spring, however this result is not confirmed.

## INTRODUCTION

Atmospheric $CO_2$ concentrations are expected to rise to double their present levels by 2100 (Watson *et al.*, 1990). In order to predict the long-term effects of elevated $CO_2$ on vegetation, reliable experimental methodologies have to be applied. Whilst the use of open-top chambers (e.g. Lawlor and Mitchell, 1991) and other field fumigation methods can be used to predict changes in plant structure and performance over time, more long-term experiments will be required. Thus, Miglietta *et al.* (1993) suggested that, elevation of $CO_2$ concentrations of 10 years or more may be necessary to establish how long-lived species, such as trees, may respond or how the genetic structure of populations of short-lived species may respond.

During the past few years, free-air $CO_2$ enrichment (FACE) studies have been used to elevate ambient $CO_2$ across large areas of plants and soil, not encumbered by chambers or other structures (Amthor, 1995). This system is based on the method used to study air pollutants on plants and ecosystems (Amthor, 1995). For example, it was applied to cotton crops in a project at the USDA-ARS, Water Conservation Laboratory, Phoenix, Arizona, U.S.A. (Hendry, *et al.*, 1988 cited in Lawlor and Mitchell, 1991). This method entailed fumigating a 23-m-diameter area with elevated $CO_2$, ensuring that there was minimal disturbance to other environmental factors. But, the technique of releasing $CO_2$ gas into the open field has problems, in that it is difficult to control concentrations and $CO_2$ costs are high (Lawlor and Mitchell, 1991). Although the FACE system has great potential, little work has been published on natural ecosystems under these conditions (Akey, Kimball and Mauney, 1988).

Researchers (e.g. Miglietta *et al.*, 1993) are exploring the possibility that natural $CO_2$ springs (surface vents of deep geothermal $CO_2$ sources) can be used as a resource (or tool ) to examine the long-term effect of rising $CO_2$ concentrations on vegetation. This method is advantageous because it is inexpensive, and naturally elevated $CO_2$ concentrations around these vents are assumed to have occurred for hundreds of years, or even longer. Normally, the vegetation around these vents have been subject to a $CO_2$ gradient, with decreasing concentration with distance from the vents (Miglietta *et al.*, 1993).

It is estimated that more than 100 geothermal $CO_2$ springs exist in central-western Italy (Miglietta *et al.*, 1993). In South Africa , exhalations of gas from the ground were noticed in an area called Alfred County, situated in southern Kwazulu Natal (Hartnady, 1985), as far back as 1922. Since that date, further occurrences have been discovered (Fig. 1). Currently, the origin of the $CO_2$ exhalations in this area is unclear, although two hypotheses have been presented. The "hotspot hypothesis" assumes that $CO_2$ exhalations occur in volcanic areas, and is of volcanic origin (Hartnady, 1985), or an alternative hypothesis suggests that acid (the origin of the acid is not known) reacts with a carbonate source (limestone rock) and releases $CO_2$. Current evidence (Stock, 1996 unpublished) show that the $\delta^{13}C$ values range between - 0.18 and -1.5 ‰, which suggests that the carbon is derived from a carbonate source ($\delta^{13}C$ of marine carbonates is ± 0 ‰ while atmospheric $\delta^{13}C$ is -8 ‰), which supports the latter hypothesis.

In order to predict the effect of elevated $CO_2$ on vegetation at a global scale, appropriate models have to be constructed. The assessment of these models, need to be tested under field conditions if they are to be used to predict the effects of global environmental change. The field experiments performed to date show different responses under elevated levels of $CO_2$. For example, observed responses to elevated $CO_2$ were small in experiments with tundra vegetation (Grulke *et al.*, 1990), but large and persistent in much warmer vegetation of a salt marsh in Maryland, USA (Ziska *et al.*, 1990). Kirshbaum (1994) used a combined model of photosynthesis (Farquar and von Caemmerer, 1982) coupled to a model of stomatal conductance (Ball, Woodrow and Berry, 1987) to test the effect of elevated $CO_2$ over a range of temperatures and $CO_2$ concentrations. He suggested that the different responses of the field experiments to elevated $CO_2$ could be due to differences in inherent $CO_2$ sensitivities at the different temperatures. Thus, field experiments provide a 'snap-shot' of vegetation response to the particular conditions prevailing at a specific location (Lawlor and Mitchell, 1991). This is why it is so important that more field experiments are performed in different regions to put together the 'pieces' of the puzzle, in order to create a global model to predict global environmental change, with future increasing $CO_2$ concentrations.

In the southern hemisphere no field experiments have been performed. Therefore, in this study, we utilise the elevated $CO_2$ spring in Natal, to assess the effect of elevated $CO_2$ on an open grassland (described in the site description below) which is located on the Bongwan gas fault. The open grassland was chosen as the study site because of its exposure to elevated $CO_2$ for decades. Furthermore, we are able to assess the effect of elevated $CO_2$ on many variables that have previously been shown to be important in other experiments. In this study the long-term effects of elevated $CO_2$ on: biomass production, $C_3$ vs.$C_4$ dominance, nutrient availability and water-use efficiency will be determined.

Elevated $CO_2$ increases the rate of photosynthesis and dry matter production of $C_3$ plants substantially but affects $C_4$ plants to a lesser extent. A review by Kimball (1983) showed that in experiments performed under a wide range of conditions, doubling of $CO_2$ increases the productivity of a large number of $C_3$ crop plants on average by 33%, while that of $C_4$ plants only increased by 10%. Another survey (Cure an Acock, 1986 cited in Bowes, 1993) showed that on average, enriched plants accumulated 30% more biomass and had a 41% greater yield. Elevated $CO_2$ studies on $C_4$ plants show mixed results: minor increases in leaf photosynthesis and growth (Akita and Tanaka, 1973, Cure and Acock, 1986) in some studies, and in others - large increases in dry matter production (e.g. Smith, 1987, Poorter, 1993).

Lemon (1983) suggests that the differences in response of $C_3$ and $C_4$ plants to elevated levels of $CO_2$ could be attributed to the differences in saturation points of $C_3$ and $C_4$ plants at present $CO_2$ levels. For $C_3$ plants growing in adequate light, photosynthesis requires 800-1000 $cm^3\ CO_2\ m^{-3}$ for saturation, while photosynthesis of $C_4$ plants, is saturated at the current atmospheric $CO_2$ concentrations (Lawlor and Mitchell, 1991). Therefore, the increase in carbon availability under elevated $CO_2$ would allow $C_3$ plants to use the carbon more efficiently during photosynthesis than $C_4$ plants. Both rising temperature and elevated $CO_2$ are expected to influence the photosynthetic rate of $CO_2$ uptake in $C_3$ plants through their direct effects at the level of primary carboxylation (Long, 1991). $CO_2$ and $O_2$ compete for the primary acceptor molecule of $C_3$

photosynthesis, ribulose biphosphate (rubP). The enzyme rubP carboxylase/oxygenase (Rubisco) catalyses both carboxylation and oxygenation (Long,1991).

Kirschbaum (1994) suggested that the enhanced photosynthetic capacity of $C_3$ plants is of immediate significance for the competition between $C_3$ and $C_4$ plants. In other words there will be a shift in species dominance under elevated $CO_2$. Increased $CO_2$ is thought to confer a selective advantage on the $C_3$ plants, increasing their competitive ability through increased shoot and root growth, and this would enhance their ability to compete with $C_4$ plants for nutrients and light. Experiments on mixed $C_3$ / $C_4$ stands have supported the idea that $C_3$ components gain an increasing biomass share of a community with increasing $CO_2$ concentration (Bazzaz and McConnaughay, 1992; Drake, 1992). The complexities of such plant / plant interactions are not consistent (Bazzaz and McConnaughay, 1992). It is important to determine the complexities of interactions between these plants to ascertain whether either C3 or C4 plants will outcompete each other under elevated CO2 (Henderson *et al.* 1995). Wray and Strain (1987) found that the "open field", competitor *Aster pilotus* Willd. ($C_3$) became more competitive with *Andropogon virginicus* L., ($C_4$) at elevated $CO_2$. Other studies of annual species grown together have demonstrated that the advantages conferred under elevated $CO_2$ on $C_3$ plants are not always as marked, and so it is difficult to predict competitive outcomes (Henderson *et al.*, 1995). Therefore, in this project the hypothesis that elevated $CO_2$ will increase the long-term the competitive ability of $C_3$ plants is tested by utilising the elevated $CO_2$ spring in Bongwan, Natal.

Smith and Epstein (1971) showed that carbon isotopic composition can be used to distinguish between $C_3$ and $C_4$ photosynthetic pathways (Ehleringer and Osmond, 1989). This technique is becoming increasingly popular in that it can be used to reconstruct past climates (White *et al.*, 1994) and determine the $\delta^{13}C$ of plant material an area, as in this study. To determine the abundance of $C_3$ and $C_4$ plants at the various sites, carbon isotope technique will be utilised. The $\delta^{13}C$ of $C_3$ plants ranges between -20 to -35‰, while that of $C_4$ plants ranges between -7 to -15 ‰ (Ehleringer and Osmond, 1989). Furthermore, this technique can also be used to trace the $CO_2$ source (Ehleringer and Osmond, 1989), in this study atmospheric $CO_2$ versus the $CO_2$ spring.

The effect of elevated $CO_2$ on plant water-use efficiency (WUE) has been well-documented in the literature. Samarakoon and Gifford (1995) have also shown that $C_4$ plants perform better with respect to water-use efficiency under elevated $CO_2$. Thus, $C_4$ plants grown in water stressed environments will be at an advantage when exposed to increased levels of $CO_2$. In this study the effect of elevated $CO_2$ on the plants WUE could affect the percentage moisture in the soil at the different sites or slope position down could be the sole determinant of soil moisture. A high percentage water content (relative to the other sites) could imply that water was utilised efficiently by the plants at that site, and vice versa.

The increased carbon gain associated with elevated $CO_2$ should increase the amount of carbon relative to other essential elements in plant tissues. Therefore, nutrient uptake might increase under $CO_2$ enrichment, however, elements such as N may not respond to the same degree as C-uptake, consequently the plant have less tissue N and improved N-use efficiency (Bowes, 1993). With the possibility that increased $CO_2$ permits a reduction of nitrogen investment in the photosynthetic machinery, it is not surprising that the percent stimulation of growth by increased $CO_2$ concentration was just as great with severely N-deficient cotton plants, compared to plants adequately supplied with nitrogen (Lemon, 1983). Phosphorus (P) uptake in plants also plays a role in determining the effect of elevated $CO_2$ on plants. This nutrient is expected to increase under elevated $CO_2$ (Stock and Midgley, 1995). With an increase in biomass, the increase in organic carbon and phosphorus, could result in an increase in mycorrhizal activity to sustain the primary productivity.

The primary focus of this study is to test the effect of elevated $CO_2$ on the plant biomass production in the grassland. The biomass production of the $C_3$ and $C_4$ plants will be assessed to determine which pathway performed better under elevated $CO_2$. The moisture content of the soils at the various sites will be calculated to determine whether the plants used water efficiently under elevated $CO_2$. Finally, nutrient analyses (N and P) of the plant and soil material at the different sites will be compared to determine the nutrient status of the plants and soil under elevated $CO_2$.

**3) Study Area**

The study area was located in the Bongwana area in southern Kwazulu-Natal, South Africa. The $CO_2$ spring is situated on Pleasantview Farm (30°40′ - 30°01′) 15km from Harding. The area consists of a large area of grassland, and the $CO_2$ gas fault running along the length of the grassland. In 1923 this area was explored for commercial exploitation, and a 27ft pipe was dug into the fault, resulting in the emission of $CO_2$ from the pipe. Laboratory analyses of the gas emitted from the pipe show that it consist of approximately 98% $CO_2$ and trace amounts of $H_2S$, with approximately more than a ton of $CO_2$ emitted per day. To identify the source of the $CO_2$ exhalation in the area, samples were analysed, and showed a $\delta^{13}C$ of between -0.18 to -1.5 ‰. The climate in the area is subtropical with approximately rain mainly falling during the summer months.

The study area consisted predominantly of *Digitaria spp.* ($C_4$), *Eragrostis spp.*($C_4$) and *Themeda tiandra* ($C_4$) with the infestation of the dicotyledenous plant *semialata.*, a $C_3$ grass, as well as some unidentified $C_3$ herbs and shrubs. The average height of the grass species was approximately one meter, while that of the dicotyledenous plants were approximately thirty centimetres. Thus the dicotyledenous plants were embedded in the grass layer.The $CO_2$ vent was located in the centre of the slope and three study sites were located, in a straight line, between the $CO_2$ vent and the top of the hill (Fig. 1). The gradient was approximately 10-15°. For the past 10 years, sugar-cane has been farmed in the area surrounding the grassland. In order to prevent biomass accumulation in the grassland area, the farmer burns the grassland (via controlled fires) every winter, thus the standing biomass is a single years production. This is done to prevent endangering the sugar-cane plantations, because in summer months the vegetation can burn easily.

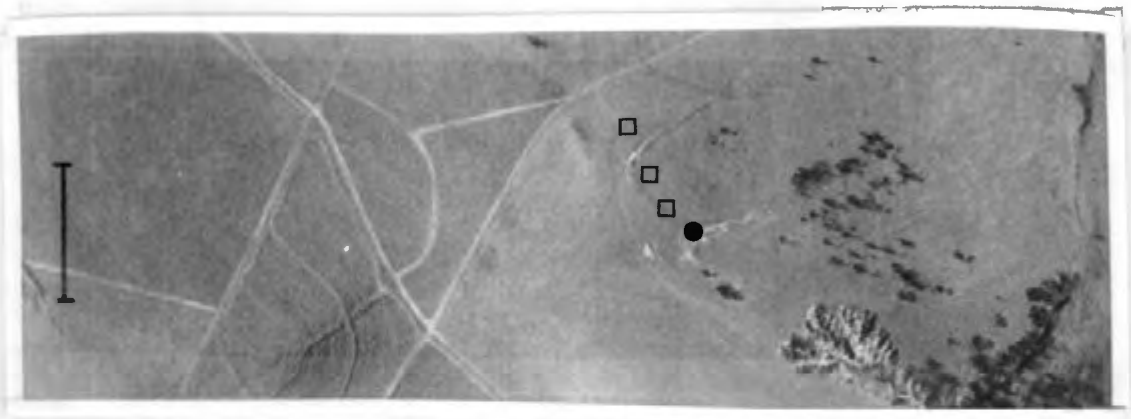


**Figure 1** : Aerial photograph showing the location of the study sites at varying distances (□, 18m, 39m and 73m) away from the CO2 source (●). Scale ?

## 4) Materials and Methods

Plant and soil material was collected in the week of the 7 - 14 May 1996. On the 8 May 1996 (at the beginning of winter), three 7 × 7½ m sites chosen 18m, 39m and 73m away from the $CO_2$ vent (Fig.1). These sites are being used for further studies of $CO_2$ effects since the $CO_2$ from the vent is now used to fumigate the lowest site (18m) via a specially constructed fumigation system.

### 4.1) Plant Material and Soil Collection

#### 4.1.1) Plant collection and biomass measurements

At each site, twenty-five 1×1m plots were laid out, and the biomass was measured for 15 plots per site. This was done by clipping by clipping each plot and dividing the plant material collected into monocotyledenous ($C_4$ and $C_3$) grasses and dicotyledenous ($C_3$) herbs. The clipped plant material was placed in separate black plastic bags, and the fresh weight was determined using a 3 decimal place mass balance. The monocotyledenous plant material was cut until homogeneous, and a representative subsample of about approximately 1/10 the fresh weight was removed and placed into brown paper bags (the fresh weight of this subsample was measured immediately). The fresh weight of the total dicotyledenous samples was taken and the material placed into brown paper bags. Samples of both categories were transported to the University of Cape Town's ecophysiology laboratory for drying, dry weight determination and chemical analysis.

### 4.1.2) Soil sample collection

Soil samples were collected outside the experimental sites (to prevent disturbance within the sites). Three samples were collected at each corner of the square experimental sites. The samples were collected 1m apart, in transects, diagonally away from the corners of the sites. Twelve soil samples were collected at each site at a depth of 20-50cm. The soil was placed into a 5×5mm vials to retain the moisture.

The soils were oven-dried at 105°C for 24 hours, and the plant samples at 80°C for 72 hours. Moisture contents were calculated for the soil and plant material after which the plant samples were ground to pass through a 40 mesh screen in a Wiley Mill (Arthur H. Thomas Scientific Apparatus, Phila, P.A. U.S.A). Ground samples were used for carbon isotope and nutrient analyses. Soils were sieved through a 2mm sieve prior to nutrient analysis.

## 4.2 Soil and Plant Material Nutrient Analysis

### 4.2.1 Soil and plant total N

Total N was determined on the soil and plant samples by micro-Kjeldahl digestion in which 1g of the dried soil sample was placed in a 25cm Kjeldahl digestion tube. One ml of distilled water, 3ml N-free concentrated sulphuric acid containing 34g $l^{-1}$ salicylic acid, a selenium-catalyst tablet and 0.2g (spatula tip) sodium thiosulphate were added. After digestion on an aluminium-block digester (carried out by leaving the tubes overnight at 150°C, increasing the temperature from 220°C to 300°C at one hour intervals and after the digest cleared, digested at 350°C for two hours) the digest was made up to 50ml with distilled water. The ammonium content was determined by the phenol-hypochlorite method (Smith, 1980).

Phenol-hypochlorite determination was carried out by adding 25ml 0.12% (w/v) EDTA, 2ml reagent A (equal parts of 0.5% (w/v) sodium nitroprusside and 10% (w/v) phenol in 95% ethanol) and 3.5ml reagent B (4 parts of alkaline phosphate buffer added to 1 part 1.5% sodium hypochlorite) to a 0.4 ml digestion solution. The solution was made up to 50 ml with distilled water and left for 60 minutes, after which the

absorbance was read at 635nm using a Bausch and Lomb Spectronic 21 spectrophotometer (Novozansky *et al.*, 1974). Three blanks and five ammonium sulphate standards in the range 0.5 to 4 μg N $g^{-1}$ ( for the soil) and 0.1 - 0.4 μg N $g^{-1}$ (for the plant material) were read simultaneously.

**4.2.2 Soil Total P**

Total P was determined on the soil and plant samples by the method modified by Grimshaw (1985) for the soil, and Murphy and Riley (1962) for the plant material.. To 0.2g of dried soil in a 50ml thick-walled boiling tube on 3.5ml of plant material; 1ml 10 $HNO_3$ : 1 $HClO_4$ : 1 $H_2SO_4$ were added. The mixture was digested on an aluminium-block digester (for 150°C for 1 hour, increasing the temperature to 250°C for 1.5 hr). The digest was cooled and diluted to 25ml with distilled water. P-determination of the plant material was done by adding 1ml of $HNO_3$ ( pre-digested for 15 minutes at 180°C, removed and cooled) to 0.1g of dried plant material, after which digestion at 180°C for 1 hour in 1ml of 10$HNO_3$: 1$H_2SO_4$: 4HClO was performed. The sample was diluted to 25ml in distilled water. A 2ml sample was used for P-molybdate determination (Welschen and Bergotte, 1994) and the absorbance was read at 700nm using a Bausch and Lomb Spectronic 21 spectrometer (Novozansky *et al.*, 1974). Three blanks and five standards set at 0 - 30 μg P $g^{-1}$ for the soil samples, and 0 - 30 μg $Pg^{-1}$ for the plant samples.

**4.3 Carbon Isotope Discrimination Analysis**

Carbon isotope analysis was performed on duplicates of each of the ground (40 mesh) monocotyledenous plant samples. In 8×5mm tin foil cups, 0.05mg of the sample was weighed out on a Sartorius 6-place microbalance, rolled into a ball and placed on a sample tray. The first row contained only duplicated blanks and standards. The standards used were Merck gelatin (Mgel) (0.05 - 0.08 mg), Nasturtium standard (NASTD) (0.05 - 0.08mg), Australian National University sucrose (ANUsuc) (0.04 - 0.06mg) and BDH glycine (0.05 - 0.07mg). Between the duplicated plant samples (0.05 -0.08mg), a single standard (NASTD) was placed. Fifty samples were combusted sequentially in a Carlo Erba NA1500 elemental analyser and interfaced via

an open-split to a Finnigan MAT 252 mass spectrometer (Bremen, Germany). The discrimination values were expressed in ‰, obtained by the following equation:

$$\delta^{13}C = (R_{sample} / R_{std} - 1) \times 1000$$

where $\delta^{13}C$ is the isotope ratio in delta units to the PDB standard, and $R_{sample}$ and $R_{std}$ are the ratios of the samples and standards respectively. Multiplying by 1000 allows expression in ‰. The standard used was PeeDee Belemite (limestone) (PDB).

### 4.4 Statistical Analysis

To determine whether there was a significant difference between the sites along the gradient away from the $CO_2$ vent, a one- way ANOVA ( Fisher, 1953) was performed. A Tukey multiple comparison test (Tukey, 1953) was performed as a post-hoc test, to determine where differences existed (Zar, 1984).

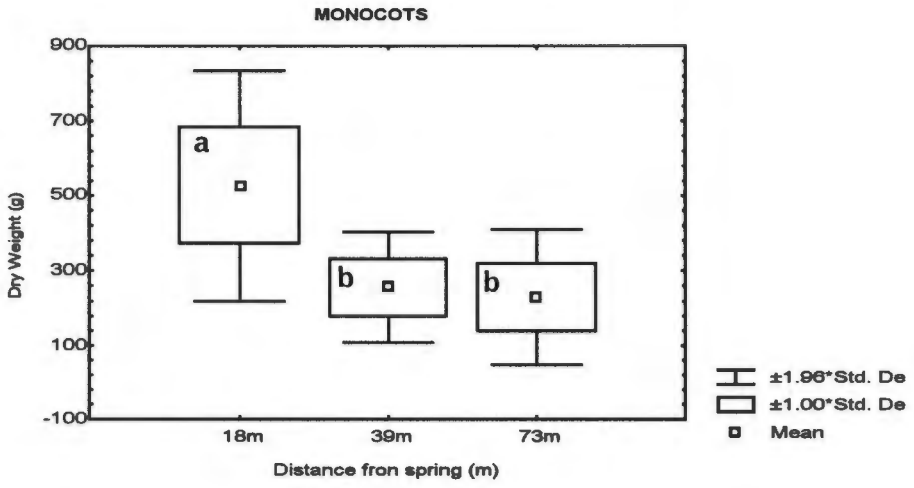
## 5. RESULTS

### 5.1 Plant Biomass

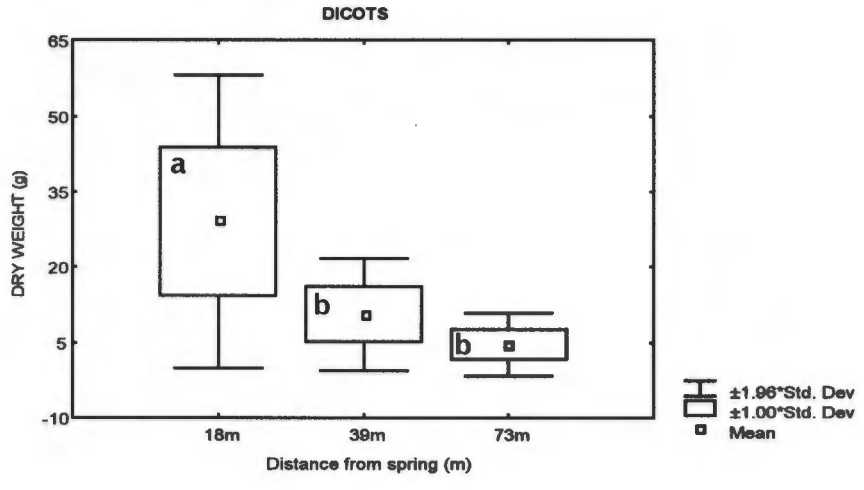
Plant biomass 18m away from the $CO_2$ spring is significantly higher ($p < 0.05$) than at the control sites (Figs. 2a and 2b). Therefore, as one moves away from the elevated $CO_2$ spring the plant biomass of both the monocotyledenous (Fig. 2a) and dicotyledenous plants (Fig. 2b) decreases.

The increase of the biomass production of the monocotyledenous and dicotyledenous plants are calculated as a ratio of the monocotyledenous: dicotyledenous plants presented in Fig 3. The dicotyledenous plants performed well at the 18m and 39m site, therefore the ratio is lower at these sites compared to the 73m site.

**2 *a*)**




**2 *b*)**




**Figures 2*a* and 2*b*** : Dry weights of the monocotyledenous and dicotyledenous plants at three sample sites along a gradient away from the $CO_2$ source (18m, 39m and 73m). One-way ANOVA analysis ($F=31.5$, $p < 0.05$ for the monocotyledenous plants and $F = 27.7$, $p < 0.05$) for the dicotyledenous plants) and differences between means are shown by different letters after Tukey HSD multiple range comparison.

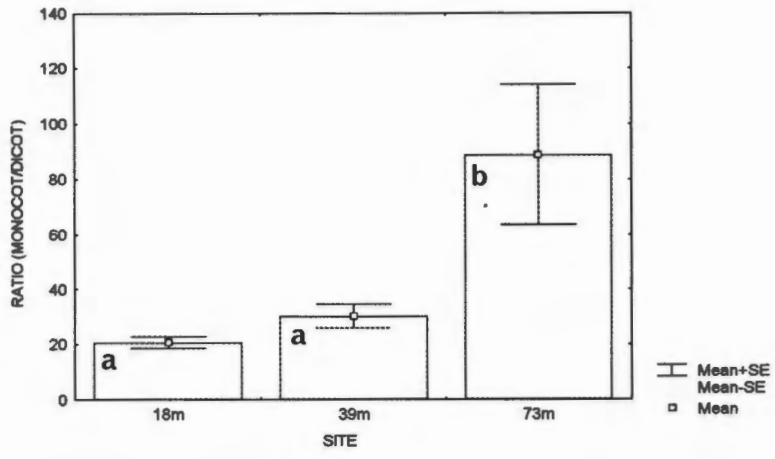



**Figure 3** : Ratios of the monocotyledenous plants to the dicotyledenous plants at three sample sites along a $CO_2$ gradient.One-way ANOVA analysis (F=6.18, p=0.004) and differences between the means are shown by different letters after Tukey HSD multiple range comparison.

## 5.2. Carbon Isotope Discrimination

Figure 4 shows a significant difference ($p < 0.05$) between the $\delta^{13}C$ values, 18m, 39m and 73m away from the $CO_2$ spring. There is no definite trend away from the $CO_2$ vent, although it was expected that the $\delta^{13}C$ at the 18m site should have the most negative possibly due to a better performance of $C_3$ plants at the elevated site (Fig. 3). The $\delta^{13}C$ discrimination value at this site contradicts the expectation since this value is highest at the site closest to the $CO_2$ vent (Fig. 4).

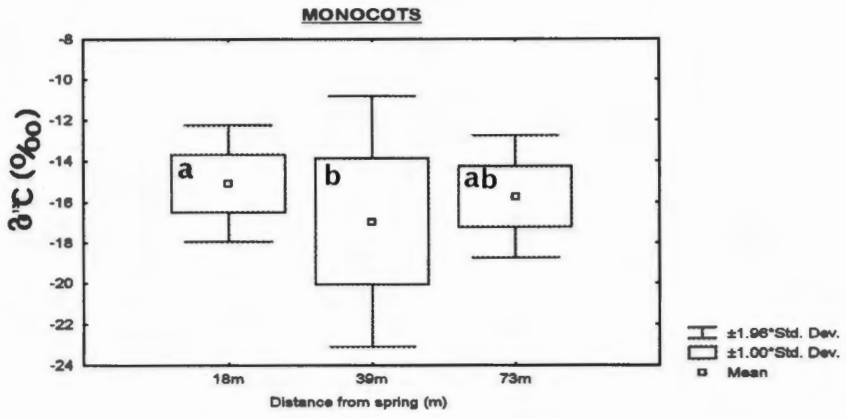



**Figure 4:** Carbon isotope discrimination values at three sample sites along a $CO_2$ gradient (18m, 39m away from the $CO_2$ source. One-way ANOVA indicate that F=4.63, $p< 0.05$ and the Tukey HSD multiple range comparisons are indicated by different letters.

## 5. 3. Soil Moisture Content (MC)

Figure 5 shows that the soil moisture content 18m away from the spring was significantly ($p < 0.05$) higher than at the sites further away from the spring. The significant difference between all the sites, ($p = 0.015$) implies that the further one moves away from the elevated $CO_2$ spring, the less water is retained in the soil because of enhanced WUE or because of differences associated with position of the sites on the slope.

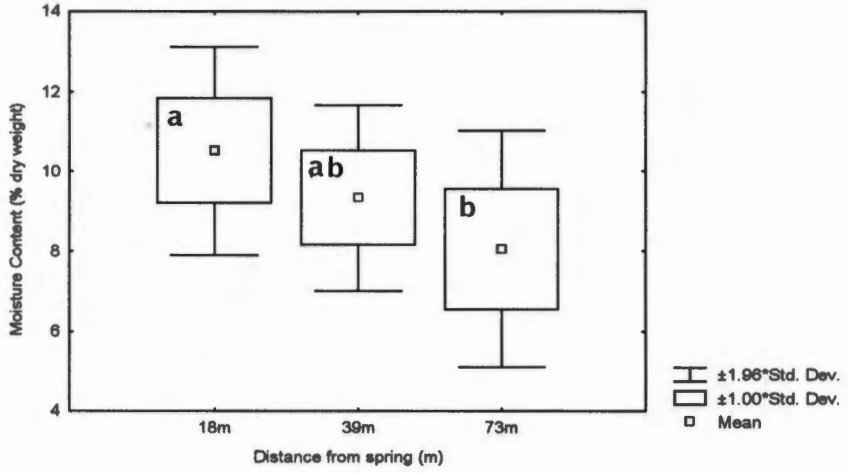


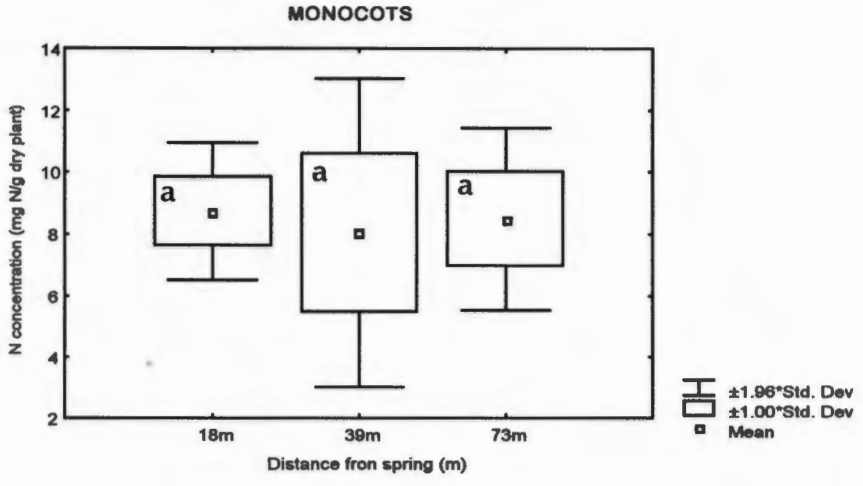

**Figure 5** : Percentage soil moisture content at (expressed on a dry weight basis) at the three sample sites (18m, 39m and 73m) along a $CO_2$ gradient. A one-way ANOVA analysis show significant differences between the means ($F=8.32$, $p=0.015$) and Tukey HSD multiple range comparisons are indicated by different letters.

## 5.4 ) Plant Total Nitrogen

There was no significant difference ($p > 0.05$) between the nitrogen contents of both the monocotyledenous and dicotyledenous plants (Figs 5a and 5b) across the different sites. Generally, the dicotyledenous plants (Fig. 5b) have lower nitrogen than the monocotyledenous plants (Fig.5a). The mean N content of the dicotyledenous plants at the elevated site was approximately 14 mg $N.g^{-1}$ dry plant, while the monocotyledenous plants had less N (approximately 9 mg $N.g^{-1}$ dry plant). Overall, the dicotyledenous plants utilised between 12 mg $N.g^{-1}$ dry plant and 15 mg $N.g^{-1}$ dry plant across all sites, while the monocotyledenous plants ranged between 7.5 mg $N.g^{-1}$ dry plant and 8.5 mg $N.g^{-1}$ dry plant. This implies that at all the sites, the dicotyledenous plants always utilised less N for photosynthesis than the monocotyledenous plants. However, there seem to be no distinct trend in the N

concentrations of the monocotyledenous or dicotyledeonous plants with increasing distance away from the spring.

**6 *a*)**

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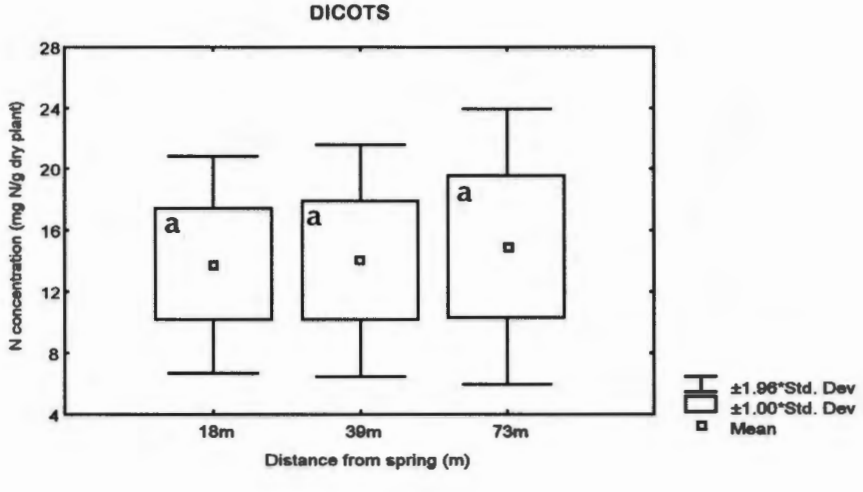



**Figure 6 *a* and 6*b* :** Plant N concentration at the three sample sites (18m, 39m and 73m) along the $CO_2$ gradient. A one-way ANOVA analysis (F=31.5, p=0.76 for the monocotyledenous plants), (F=0.16, p=0.84 for the dicotyledenous plants) A Tukey multiple range comparison are indicated by letters.

## 5. 5 ) Soil Total Nitrogen

There is no significant differences (p = 0.05) between the N concentrations of the soil at the different sites (Fig. 7) and values range between 1.5 and 2.5 mg $N.g^{-1}$ dry soil (Fig. 7). The higher concentration of N at the site nearest to the spring (18m) suggests

that there is a difference in N pool sizes and possibly cycling between sites, but this is not statistically significant.

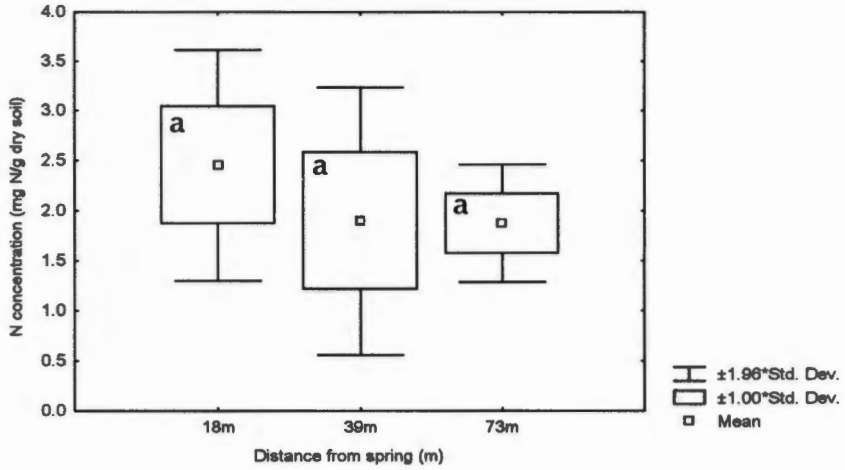
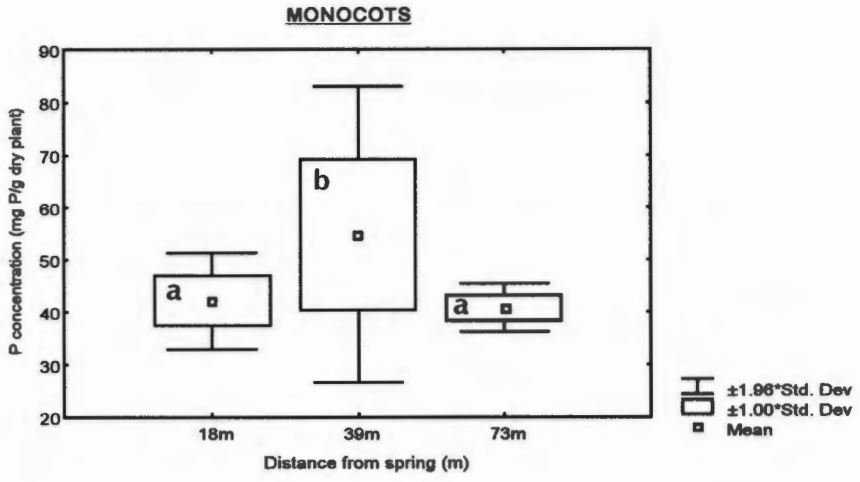



**Figure 7:** Soil N concentration at three sample sites (18m, 39m and 73m) along a $CO_2$ gradient. One-way ANOVA indicate F=3.27, p=0.05, and a Tukey HSD multiple range comparison are indicated by letters.

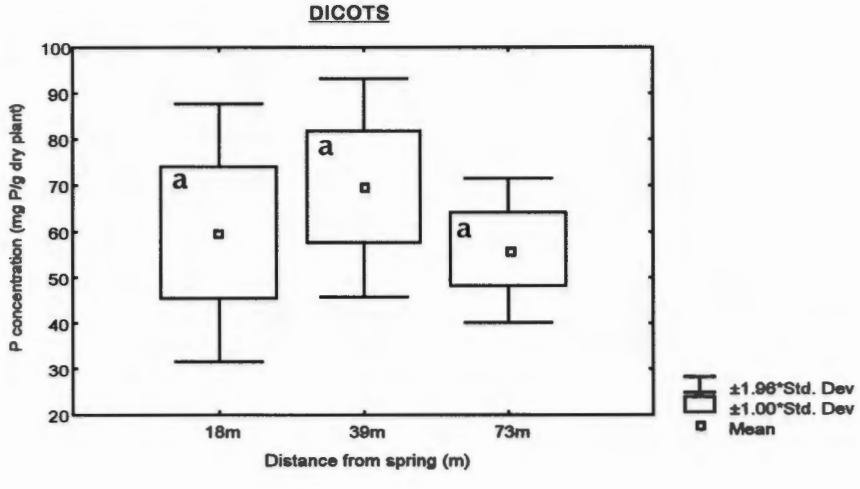
## 5. 6 Plant Total Phosphorus

Figures 8a and 8b show the total phosphorus concentrations of the monocotyledenous and dicotyledenous plants respectively. There is a significant difference between the phosphorus concentration of the monocotyledenous plants (p=0.015) (Fig. 8a) accross the sites, while there is no significant difference between the P concentrations of the dicotyledenous plants (p=0.76) (Fig. 8b). Although there is no significant difference between the P concentrations of the dicotyledenous plants across the sites, both the monocotyledenous and dicotyledenous plants show the same trend. That is the highest P concentration at the site 39m away from the $CO_2$ source, with a decrease in value at the 18m site, and finally a further decrease in P concentration at the 73m site.

**8 *a*)**




**8 *b*)**




**Figures 8*a* and 8*b*:** P concentrations of the monocotyledenous and dicotyledenous plant material One-way ANOVA show that F=5.33, p=0.015 for the monocotyledenous plants, and F=0.27 and p=0.76 for the dicotyledenous plants. The Tukey HSD test is indicated by letters.

## 5.7 ) Soil Total Phosphorus

The phosphorus concentration of the soil shows no significant differences between the sites (Fig. 8). There is however a trend in that the P concentration increases as one moves toward the $CO_2$ spring, but this trend is not statistically significant (p=0.05).

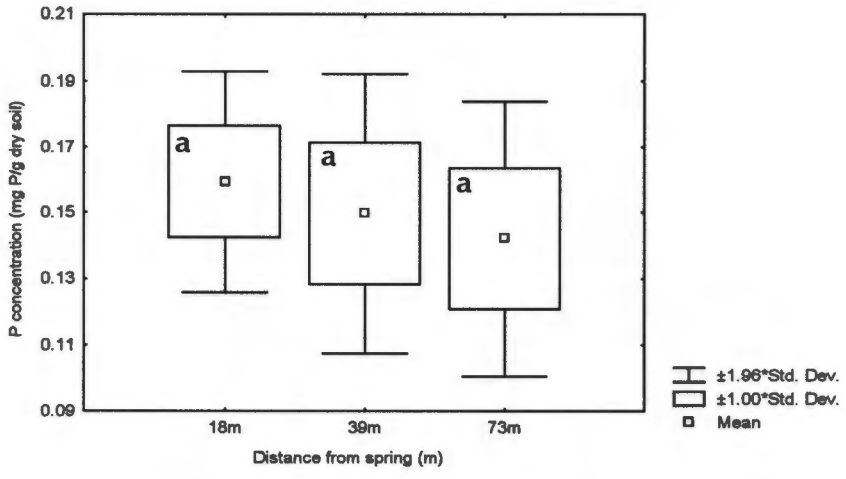



**Figure 9:** P concentration of the soil. A one-way ANOVA indicates F=1.46 and p=0.25. The Tukey HSD multiple range comparison is indicated by letters.

## 6. DISCUSSION

In this study, the effect of elevated $CO_2$ on a grassland biomass production was assessed in an area in which $C_4$ plants are the characteristic species. Carbon isotope analysis on the monocotyledenous plants (Fig. 4) and dicotyledenous plants (data not shown) of the area, confirm that the monocotyledenous plants were predominantly $C_4$, while the dicotyledenous plants were predominantly $C_3$ plants.

Many authors have assessed the effect of elevated $CO_2$ on the biomass production of $C_3$ and $C_4$ plants (Kimball, 1983 and Poorter, 1993). It has generally been found that $C_3$ plants respond to elevated $CO_2$ by increasing their biomass production, while $C_4$ plants shows little or no response. In this experiment, biomass production at the elevated site (18m) increased significantly ($p< 0.05$) relative to the control sites (39m and 73m) (Figs. 2a and 2b).

Researchers (Bowes, 1991; Lawlor and Mitchell, 1991, Stitt, 1991) propose that a possible reason for the growth stimulation of $C_3$ plants under elevated $CO_2$, could be the increase in activity of rubisco. In $C_3$ plants rubisco has low catalytic activity, operates below $K_m$, and is inhibited by $O_2$ (Bowes, 1991). At the $CO_2$ spring, the long-term exposure of the plants to $CO_2$ could have caused an increase in the $CO_2$ / $O_2$ ratio (Lemon, 1983) which resulted in a subsequent stimulation of $C_3$ photosynthesis and inhibition of photorespiration (see Amthor, 1995). Thus, productivity of the $C_3$ plants was enhanced.

The monocotyledenous plants in this study also responded to elevated $CO_2$ by increasing their biomass production, which is inconsistent with most literature (Poorter, 1993). Theoretical evidence (Lemon, 1983) suggests that $C_4$ plants shouldn't respond to elevated $CO_2$ since these plants are currently saturated at the present day $CO_2$ concentrations (Lawlor and Mitchell, 1991). $C_4$ plants possess a $CO_2$ - concentrating mechanism at the site of rubisco and therefore often do not show a response in their rate of $CO_2$ fixation with doubling of the ambient $CO_2$ concentration (Bowes, 1991). In a compilation of literature sources Poorter (1993) concluded that the results obtained show that the effects of elevated $CO_2$ on $C_4$ plants are marginal.

For example, Poorter (1993) reported a significant average increase ( 22%) in dry matter accumulation of $C_4$ plants, compared to the 41% increase for $C_3$ plants. Furthermore, in experiments with "mini-communities" Zangerl and Bazzaz (1984) found that $C_4$ species increased their biomass and seed production. While Bowes (1993) showed that $C_4$ plants did not respond to elevated $CO_2$.

It must be noted, however, that the $C_3$ grass *Allorteropsis seminala,* could have contributed to the increase in biomass - due to the invasion of this plant with other $C_4$ grasses. This plant was not known to be a $C_3$ grass at the time of sampling (this species has both $C_3$ and $C_4$ subspecies) and was assumed to be a monocotyledenous plant during sampling. Later carbon isotope tests performed on the plant showed that it was a $C_3$ grass. However, the increase in biomass of the monocotyledenous plants should not only be attributed to the invasion of *Allorteropsis seminala per se,* since the carbon isotopic composition shows a very strong $C_4$ signature. Other factors could contribute e.g. the decrease in stomatal conductance (Gifford, 1984, Samarakoon and Gifford, 1995) and increase in water-use efficiency of the plants, which is confirmed by the soil moisture content results (Fig. 5). These results show that the soil moisture content at the elevated site is significantly higher ($p < 0.05$) than the control sites (however, we are not certain whether the increase in soil moisture content as one approaches the spring is due to more water being retained by the soil and enhanced WUE or because of differences associated with position of sites on the slope). Another factor could be that some authors (Poorter, 1993) believe that perhaps $C_4$ plants are not saturated at present day $CO_2$ levels, and therefore do utilise excess $CO_2$. Furthermore, respiration and C allocation can play a role in the increase in productivity of $C_4$ plants, these aspects were not covered in this study, but could be performed in future studies.

The significant ($p < 0.05$) decrease of the ratio of the monocotyledenous plants to dicotyledenous plants 18m site (Fig. 2), implies that the dicotyledenous plants performed better at this site than at the other two sites. In other words, the dicotyledenous plants could have invaded this site, due to their enhanced performance

under elevated $CO_2$ (Lemon, 1983; Bowes, 1991). Kirschbaum, (1994) suggested that the enhanced photosynthetic capacity of $C_3$ plants is of immediate significance for the competition between $C_3$ and $C_4$ plants. In competition experiments involving $C_3$ and $C_4$ stands, similar results were obtained by some authors (Patterson *et al.*, 1984; Wray and Strain, 1987), but in contrasting results were obtained by others (Henderson *et al.* 1995). The carbon isotope signature in Figure 4 should have been the most negative at the 18m site due to the invasion of dicotyledenous plants at this site, but this result is the most positive. If the result was as expected (i.e. the most negative at the elevated site), this would confirm that $C_3$ plants did invade at this site. However, this contrasting result could be due to experimental error or due to the varying $CO_2$ sources at the different sites.

The long-term exposure to elevated $CO_2$ at the spring would make one expect that the $C_3$ plants would dominate the area. This contrasting result could be due to the burning of the grassland every season, in which the $C_3$ plants first have to recruit and establish itself before it could dominate in the area or the acclimation response (Midgely *et al.*, 1995; Amthor, 1995) which limits photosynthesis in plants. However, if this is examined at the physiological level, it could be accounted for by the acclimation response of photosynthesis which could be explained by the a possible increase in starch accumulation in the leaves. The non-significant increase in N concentration of the monocotyledenous plants at the elevated site (Fig. 6a), could be due to the apparent starch accumulation in the leaves (Poorter *et al.*, 1988).

The non-significant ($p>0.05$ ) increase in N concentration of the dicotyledenous plants at the elevated site, is probably due to no acclimation response, which can be explained by starch accumulation in the leaves. Many plants show an increase in leaf starch content on long-term (days to months) exposure to elevated $CO_2$ (Farrar and Williams, 1991). For example, *Plantago major* (Poorter *et al.*, 1988) showed this response. High starch accumulation inhibits photosynthesis (Nafziger and Koller, 1976 cited in Poorter *et al.*, 1988). If the starch is not utilised (i.e. transported to appropriate sinks), acclimation results. However, if an appropriate sink exists, the 'additional' starch will be utilised (Cure *et al.*, 1987 cited in Farrar and Williams, 1991). Another possible

reason why $C_3$ plants did not dominate the area at the experimental site, could be due to a combination of other factors e.g. temperature, light and nutrient availability interacting at the canopy level, making the effect of increased $CO_2$ less important on plant productivity (Farrar and Williams, 1991).

Nutrient supply to the plants could play a role in affecting plant productivity under elevated $CO_2$. Figures 6a and b, 7 , 8a and b and 9 show the nitrogen concentration in the monocotyledenous plants, dicotyledenous plants and soil. The nitrogen concentration of the foliar constituents as well as the soil, showed no significant increases. The dicotyledenous plants (Fig.7 ) show results consistent with the literature (Conroy, 1992), in that a decrease in the N concentration at the elevated site is expected. The monocotyledenous plants (Fig. 6) however, show contrasting results. Decreases in nitrogen in response to elevated $CO_2$ have been attributed to a reduced flux of nitrogen through the photorespiratory cycle as well as to a decline in concentrations of Rubisco or other enzymes of the photoreductive cycle (Conroy, 1992). In the monocotyledenous results (Fig. 6a), the higher concentration of N at the elevated site could imply that the monocotyledenous plants did not respond to elevated $CO_2$ and that the significant increase in dry weight at the elevated site (Fig. 2a) could be due to the invasion of *Alloteropsis seminala.* But, due to no significant increase in N concentration at the elevated site, the possibility that the monocotyledeous plants responded to elevated $CO_2$ still exists. However, the response of the N concentration of the dicotyledenous plants (Fig.6b), although not significant, is consistent with the literature. The results confirm that the $C_3$ plants are responding to elevated $CO_2$. The N concentration in the soil (Fig.7) is higher at the elevated site compared to the control sites. The availability of N in the soil may have increased because of better litter quality.

Another important nutrient which will respond to elevated $CO_2$, is phosphorus (Figs. 8a and b, 9). Phosphorus may be required in the same or larger quantities under elevated $CO_2$ conditions because the foliar nutrient requirements necessary to sustain maximum photosynthetic rates are increased (Stock and Midgley, 1995). This was not the case with regard to the monocotyledenous plants (Fig. 8), which show a significant decrease

in P concentration at the elevated site compared to the control sites. Thus, these results contradict the literature. The significant decrease in P concentration at the elevated site of the monocotyledenous and dicotyledenous plants (Figs. 8a & 8b) could be attributed to the elevated site not utilising the available phosphorus because the photosynthetic rates did not increase - this could be due to the acclimation effect in photosynthesis (Amthor, 1995). So, the phosphorus was not needed for photosynthate transport because inorganic phosphate fluxes into the chloroplast, due to no increase in carbon via the photoreductive cycle. However, the P concentration of the soil (Fig. 9) showed an increase at the elevated site, although this was not significant. This could be due to the increase in fine root or mycorrhizal growth (Norby *et al.* 1986), or if the nutrient supply increases through the stimulation of biological activity in the soil and rhizosphere (Conroy, 1992 ).

However, it should be pointed out that in the majority of $CO_2$ - enrichment studies, the foliar nutrient concentrations have not been measured. Even when they were the significance of the data could be doubtful because the levels required to produce maximum growth at elevated $CO_2$ have not been determined. Therefore, in this study, the contrasting results shown by the N and P concentrations at the elevated sites could be meaningful because it could imply that the long-term exposure of plants to elevated $CO_2$ has little effect on the nutrient status of the vegetation. This could be due to the enhancement of other factors that mask the $CO_2$ effect e.g. light, temperature, wind speed and edaphic factors. In addition to this, Stock and Midgley (1995) state that a knowledge of what precise changes in nutrient concentrations of plants exposed to elevated $CO_2$ occur is essential for the development of successful decomposition models which will allow for a better understanding of nutritional effects at the ecosystem level. It is therefore evident that more experimentation is required in this area of research.

## CONCLUSIONS

The results of this study show similarities with some studies ( e.g. biomass production, soil moisture contents and C-isotope results) and differences (e.g. P and N concentrations) with other studies. There are certain aspects of the experiment in

which further experimentation is required. For example, the soil moisture contents which show an increase in water use efficiency and the nutrient availability experiment. Nevertheless, this study establishes that growth enhancement from $CO_2$ enrichment exists at the canopy level. Thus, the use of $CO_2$ springs as a tool to predict plant productivity is essential. The method has potential in that the temperature-specific increase in $CO_2$ concentration could be addressed in various parts of the world by incorporating a FACE project in most areas of the world.

## FUTURE RESEARCH

This study is a preliminary study which is presently being done collaboratively with the Agricultural research council (ARC), National Botanical Institute (NBI) and led by Professor William Stock of the University of Cape Town. Future experiments will address mycorrhizal activity, plant destruction and recruitment and continued biomass studies. The duration of this experiment will be three years.

## ACKNOWLEDGEMENTS

I would like to thank Professor Willy Stock for his continual guidance and encouragement throughout this project.

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